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(54) Title: METHOD FOR DIAGNOSIS OF MONOCLONALITY IN LEUKAEMIA AND LYMPHOMA

(57) Abstract

A method for the detection of lymphoid leukaemia and/or lymphoma in a tissue sample comprises determination of homogeneity or heterogeneity of the length of immunoglobulin and/or T-receptor gene segments in the sample to indicate the presence or absence of monoclonality of the B- and/or T-lymphocyte population in the sample. The gene segment may be amplified, then size separated prior to length determination. A kit for performance of the method is also disclosed.

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METHOD FOR DIAGNOSIS OF MONOCLONALITY IN LEUKAEMIA AND LYMPHOMA

This invention relates to a diagnostic method which may be used in leukaemia and lymphoma for detection of malignancy and determination of its lineage.

Lymphocytes, the cells responsible for immunity, are of two types:

(a) the B-lymphocytes, each of which produces a specific immmunoglobulin molecule which attaches to a particular foreign antigen; and

(b) the T-lymphocytes, each of which has a specific surface receptor which enables the cell to attach to a particular foreign antigen.

Each B-lymphocyte contains a unique immunoglobulin gene which differs moderately in structure from the immunoglobulin gene of all other B-lymphocytes and markedly in structure from the immunoglobulin genes of all

other body cells, including T-lymphocytes. Similarly, each T-lymphocyte contains a unique T-receptor gene which differs moderately in structure from the T-receptor gene of all other T-lymphocytes and markedly in structure from the T-receptor gene of all body cells, including B-lymphocytes.

Lymphoid leukaemias and lymphomas are a form of cancer of the lymphocyte tissue. Each leukaemia or lymphoma arises from a single B- or T-lymphocyte which multiples, spreads and eventually results in death unless treated. As all the tumor cells are descended from a single cell, they are all genetically the same and all will contain the same unique gene, a unique immunoglobulin gene if the tumour arose in a B-lymphocyte, or a unique T-receptor gene if the tumour arose in a T-lymphocyte.

Conversely, if a tissue is suspected as being involved by leukaemia or lymphoma, the detection of a unique immunoglobulin gene is presumptive evidence of a tumour of B-lymphocytes whereas detection of a unique T-receptor gene is presumptive evidence of a tumour of T-lymphocytes.

The object of the present invention is to determine whether or not leukaemia or lymphoma is present in a tissue sample by determining whether or not a monoclonal B- or T-lymphocyte population is present in the sample. In general terms, the invention does this by focussing on a discrete segment of the immunoglobulin or T-receptor molecule and determining whether all or most of these segments in the tissue sample are rearranged and have precisely the same length, implying that they are derived from the same unique molecule.

Present methods for detection of monoclonality are based on restriction enzyme digestion, followed by Southern blotting and gene probing. This approach is complex, expensive and time consuming, so that information is usually provided too late to be of substantial practical value.

By contrast, the method of the present invention provides a rapid and sensitive diagnostic test. In addition, it is also very versatile as material which can be used as the tissue sample includes blood, tumour tissue in node or bone marrow, formalin-fixed embedded histological material, aspirated cytological material or cells on slides.

According to the present invention, there is provided a method for lymphoid leukaemia and/or lymphoma in a tissue sample, which comprises the determination of the homogeneity or heterogeneity of the length of immunoglobulin and/or T-receptor gene segments in said tissue sample to indicate the presence or absence of monoclonality of the B- and/or T-lymphocyte population in said sample.

According to one embodiment of the present invention there is provided a method for the detection of monoclonality and presumptive malignancy in a tissue sample, and/or for the determination of the B-lymphocyte or T-lymphocyte origin of a tumour, which comprises the steps of:

(a) amplification of immunoglobulin and/or T-receptor gene segments in said tissue sample by means of the polymerase chain reaction using specific primers or mixtures of primers for said immunoglobulin and/or T-receptor gene segments; and

(b) size separation of the amplified segments to determine homogeneity or heterogeneity of the length of the amplified segments.

In one particular embodiment, the primers used are consensus primers for the immunoglobulin and/or T-receptor gene segments.

The present invention also extends to a kit for performance of the method of the invention as broadly outlined above, comprising a set of specific primers or mixtures of primers for immunoglobulin and/or T-receptor gene segments for amplification of immunoglobulin and/or T-receptor gene segments in a tissue sample by the polymerase chain reaction.

Once again, in a particular embodiment, the primers used are consensus primers for the immunoglobulin and/or T-receptor gene segments.

The immunoglobulin (Ig) and T-receptor (Tr) genes are present in all cells. Each consists of 4 families, the variable (V), diversity (D), joining (J), and constant (C) region family (except for immunoglobulin light chains which lack a D segment). In the process of development of a B- or T-lymphocyte, the gene is rearranged so that one randomly chosen member of each family is joined together to form the final molecule. Random mutations also occur at the VD, DJ and JC joining points. As a result of this random joining and mutation, the final immunoglobulin or T-receptor molecule is virtually unique. However, there are two features of particular importance with regard to the present invention:

(a) The number of bases removed and/or inserted at the VD, DJ and JC junctions is quite variable, so that different immunoglobulin or T-receptor molecules, particularly the segments spanning VD, DJ and JC junctions, differ substantially in length.

(b) There are some regions of similarity if not of absolute identity within the genes. These comprise certain parts of the V regions, termed "framework" regions, and parts of the J and C regions.

The present invention involves the use of the polymerase chain reaction (PCR) in a new way. The PCR is now well known, having been first described in 1985, and it enables the exponential amplification of small cDNA segments provided that their sequence is known. The principle of the PCR is that two DNA primers, one for each DNA strand, are used in successive cycles of denaturation followed by DNA synthesis and this results in exponential amplification of the segment of DNA bounded by the primers. As each cycle takes 6-7 minutes, it is practical to perform 20-30 cycles, and as each cycle may be up to 70-80% efficient, there can be amplification of up to 10^6-fold at high fidelity.

The PCR as described is based on primers, each exactly complementary to a known sequence. In one embodiment of the present invention, the problem of amplifying immunoglobulin and T-receptor molecules has been solved by utilizing consensus primers to regions which have a similar but not identical sequence in the immunoglobulin and T-receptor genes respectively. These regions comprise the framework portions of the V regions of the immunoglobulins, conserved V regions of the T-receptor genes, and parts of the D,J and/or C regions of

the immunoglobulin or T-receptor genes. Primers to these regions work for two reasons. Firstly, they have a structure which is sufficiently homologous to the appropriate region of the immunoglobulin or T-receptor DNA or RNA to recognise only those regions in total DNA or RNA. Secondly, the PCR region will only work if the sequence recognised by the two primers is short. This condition only obtains for those segments of DNA or RNA in which the V, D and J (or C) regions have been joined together and a mature immunoglobulin or T-receptor molecule has been formed. As a result of these two properties, the primers will recognise and amplify only the final mature immunoglobulin or T-receptor molecule.

Use of these primers in the PCR will result in amplification of the inter-primer segments of all mature immunoglobulin or T-receptor molecules in a tissue sample. The amplified segments will cross the VD, DJ (and JC) junctions. As a result the final amplified piece of DNA, irrespective of its sequence, will have a single length if all of the immunoglobulin or T-receptor genes in the tissue are derived from a monoclonal, malignant population or will have a heterogenous length if the molecules are derived from a heterogenous non-malignant population. Primers for the immunoglobulin molecule will identify malignant populations of B-lymphocyte origin, primers for the T-receptor molecule will identify malignancy of T-lymphocyte origin.

The lengths of the amplified pieces of DNA can be simply determined by separating the DNA molecules by a technique which separates molecules on the basis of size. At present electrophoresis in agarose or polyacrylamide gel is used but chromatography would have advantages owing

to the feasibility of automation. The size separated molecules can be identified by non-isotopic means, e.g. ethidium bromide staining, by radioisotope incorporation, or by Southern transfer and hybridization to an internal probe.

Further features of the present invention will be apparent from the following detailed description.

A. SYNTHESIS OF PRIMERS

1. Primers for the Immunoglobulin Gene

The 4 regions of DNA sequence which are similar in all immunoglobulin heavy chain genes are:

(a) in the 3' end of the J-region, 30 of 34 bases are conserved in all six germ-line J-chains. 3 DNA primers which will stick to the (+) strand DNA have been used:

ELJ_H-5'TGAGG AGACG GTGAC CAGGG TNCCT TGGCC CCAG3'

LJ_H- 5'TGAGG AGACG GTGAC C 3'

VLJ_H-5' GTGAC CAGGG TNCCT TGGCC CCAG3'

(wherein N represents any of the four nucleotide bases.)

(b)-(d) the V-region can be subdivided into 3 "framework" regions and intervening "chain determining" regions. The "framework" regions are similar in all immunoglobulins; the "chain determining" regions vary greatly. By comparing sequence of 17 lymphocyte lines, conserved stretches of DNA have been identified in each framework region. However, the sequences are not highly conserved. As it is not clear how mis-match will affect the PCR, redundancies have been introduced into some synthetic primers. Primers which will hybridize to (-) strand of DNA include:

FR1BR: 5'CT[C/G][T/A]C CTG[T/C][G/A]CAG[T/C]C TCTGG 3'
FR1BS: 5'CTCTC CTGTG CAGCC TCTGG 3'
FR2A: 5'TGG[A/G]T CCG[C/A]C AG[G/C]C[T/C] [T/C]CNGG 3'
FR3A: 5'ACACG GC[C/T][G/C]T GTATT ACTGT 3'

Primers which stick to the (+) strand of DNA include:

FR3A-: 5'ACAGT AATAC A[G/C][A/G]GC CGTGT 3'

Primers which stick to the opposite strand of DNA and/or for the other immunoglobulin chains could equally well be used.

Primers modified to contain internal or terminal restriction enzyme sites have also been used successfully. They aid subsequent cloning.

2. Primers for the T-receptor Gene

Analogous primers are used for the T-receptor genes. The principles applied in selecting primers for these genes are the same as for the immunoglobulin gene but there are some differences of emphasis. In particular, there is more emphasis on generation of diversity by removal of nucleotides and insertion of random nucleotides at VD, DJ and JC junctions. The V-regions of the T-receptor cannot be divided easily into less variable and "framework" and more variable "chain determining" regions. However, near the VD junction a stretch of about 20 bases is conserved.

(a) T-receptor alpha chain

JAT1 5' TTC CAA AGA TCA G(T/C)T TG 3'
JAT2 5' A(A/G)C CTG GT(T/C) CCT (T/G)(T/G)T CAA A3'
3VTA 5' GA(C/T) TCA GC(T/C) GTG TAC (T/C)(T/A)(C/T) TG 3'
5VTA1 5' (A/T)AC (C/T)T(A/c) (C/T)TC TGG TA(C/T) AA(A/G) CA 3'

CTA 5' GAA TAG GCA GAC AGA CTT GT 3'
PTA 5' ACT GGA TTT AGA GTC TCT 3'

JAT2 is the J-region primer for the PCR.
JAT1 is an internal probe.
3VTA is the V-region primer for the PCR, about 80 base pairs from JAT1.
5VTA is a V-region primer 200-300 base pairs from JAT1.
CTA and PTA are within the constant region.
The V-and J-region primers are consensus primers.

(b) T-receptor beta chain

JBT1 5' CG GGT (G/C)CC T(T/G)G CCC (G/A)AA 3'
JBT2 5' AG CAC GGT GAG CC(G/T) (G/T)GT (G/C)CC 3'
3VTB1 5' GAC (T/A)(C/G)A (G/A)(G/C)C GTG TAT CT(C/T) TG3'
3VTB2 5' CAG ACA TCT CTG TAC (C/T)TC TGT GCC 3'
5VTB1 5' C(A/T)(C/A)(T/C)(A/C) T (T/G)T(A/T)(T/C) TGGTA CCGAC AG 3'
5VTB2 5' TGTAC TGGTA TCGAC AAG 3'
CTB 5' TTT GGG TGT GGG AGA TCT CTG C 3'
PTB 5' CTT CTG ATG GCT CAA ACA 3'

JBT2 is a J-region primer for the PCR.
JBT1 is an internal probe.
3VTB1 and 3VTB2 are V-region primers about 80 base pairs from JBT1, for TCR beta subclasses $Beta_1$ and $Beta_2$ respectively.
5VTB1 and 5VTB2 are V-region primers 200-300 base pairs from JBT1, for TCR beta subclasses $Beta_1$ and $Beta_2$ respectively.
CTB and PTB are within the constant region for all Beta genes.

The V- and J-region primers are consensus primers.

(c) T-receptor gamma chain

JGT12-	5' AAG TGT TGT TCC ACT GCC AAA 3'
3VTG	5' GTC TAT TAC TGT GCC ACC TGG 3'
CTG	5' TCT GGA GCT TTG TTT CAG CAA 3'
PTG	5' GGA AGA AAA ATA GTG GGC 3'

JGT12 is a J-region primer for the PCR. It is based on the sequence of two of the four known J-chains of TCR class Gamma.

3VTG is a V-region primer about 80 base pairs from JGT12. CTG and PTG are within the constant region.

As constant region primers are sometimes used for amplification, depending on the individual case, it may be necessary to precede the PCR amplification by starting from RNA and carrying out one round of reverse transcription.

The internal probes JAT1 and JBT1 described above can be used for final detection of an amplified product if it cannot be visualized as an ethidium - stained band. The probe can be labelled, usually radioactively, and thereby provides a very sensitive and specific method for detection of the amplified product.

An alternative approach applicable to the gamma chain (Tg receptor) is to use a mixture of primers for the 9 V-regions and 5 J-regions. Sequences used are:

V-regions

Primer	Sequence
TCRVG2PST	5' CTTC CTGCAG ATG ACT CCT ACA ACT CCA AGG TTG 3'
TCRVG3PST	5' CTTC CTG CAG ATG ACG TCT CCA CCG CAA GGG ATG 3'
TCRVG4PST	5'CTTC CTG CAG ATG ACT CCT ACA CCT CCA GCG TTG 3'
TCRVG5PST	5' TTC CTG CAG ATG ACG TCT CCAA CTC AAA GGA TG 3'
TCRVG8PST	5' CTTC CTGCAG ATG ACT CCT ACA ACT CCA GGG TTC 3'
TCRVG9PST	5' GG(A/G/C/T) ACTG CAG GAA AG GAA TCTG GCATT CCG 3'
TCRVG10PST	5' CT CTG CAG AAT CCG CAG CTC GAC GCA GCA 3'
TCRVG11PST	5' CA CTG CAG GCT CAA GAT TGC TCA GGT GGG 3'
TCRVG12PST	5' ACT CTG CAG CCT CTT GGG CAC TGC TCT AAA 3'

J-regions

Primer	Sequence
JGT_{12}-	5' AAG TGT TGT TCC ACT GCC AAA 3'
JGT_3	5' AGTTA CTA TG AG C (T/C) T AGT CCC 3'
JGT_4	5' TGT AAT GAT AAG CTT TGT TCC 3'

The V-region primers contain 20-24 bases (those nearest the 3' end) which match the target precisely. They also contain, near the 5' end, the sequence CTGCAG, as site for a restriction enzyme. This site will aid subsequent cloning, should cloning be necessary.

B. AMPLIFICATION OF DNA

Both DNA or RNA may be used. cDNA is made from RNA using reverse transcriptase and one or both PCR primers. The cDNA is precipitated and resuspended in water and the PCR reaction then used. Approximately 30 cycles of the reaction are performed using polymerase from Thermus aquaticus. Products of the reaction are separated on agarose gels and become visible following staining with ethidium bromide. Southern blotting and hybridization to a DNA probe which binds internal to the primer binding sites is also usually performed. Control size-markers aid in determining size of the amplified segment.

C. RESULTS OF TESTING FOR MONOCLONALITY USING CONSENSUS IMMUNOGLOBULIN PRIMERS

Tables 1 and 2 set out the results of testing various samples for monoclonality using the immunoglobulin gene only.

TABLE 1 Results on Extracted DNA

Material	Number	Result
Normal T cell clones	15	no amplification
Comment: As predicted.		
Normal B cell clones	16	1 or 2 discrete bands
Comment: As predicted.		
Normal mixed blood lymphocytes	20	diffuse peak
Comment: As predicted.		
B-cell lymphomas	24	1 or 2 discrete bands in 19*, no amplification in 5**
Comment: *As predicted. ** The cases showing no amplification probably represent cases with an unusual form of rearrangement.		

TABLE 2 Results on Fixed Material and Node Aspirates

Material	Number	Results
Fixed material		
B cell lymphomas	26	1 or 2 discrete bands in 24.
T cell lymphomas	7	no amplification
Reactive nodes	9	no amplification
Carcinomas	12	no amplification
Node aspirates		
B cell lymphomas	5	1 or 2 discrete bands in 3
Carcinomas	2	no amplification.

These results were obtained using primers LJ_H^- and FR3A described above. Thirty cycles of amplification were performed and the amplified fragments were electrophoresed in 2% agar and stained with ethidium bromide to enable visualisation with ultraviolet light. Similar results have been obtained by adding radioactive nucleotide (CTP) for the last five cycles of amplification, electrophoresing, and visualising the fragments by autoradiography.

D. RESULTS OF TESTING FOR MONOCLONALITY USING T-RECEPTOR PRIMERS.

Using the same PCR procedures as described above with one or other pairs of consensus primers, a discrete rearrangement has been detected using one or other pair of primers in 6 of 8 normal T-cell clones.

TABLE 3 Results on 8 normal T-cell clones using consensus primers.

Primer combination	Number of clones showing a discrete band
3VTA & JAT2	3
3VTA & JBT2	5
3VTB2 & JBT2	2
5VTB1 & JBT1	5
5VTB2 & JBT2	4

Similarly, using a mixture of all 9 V-region primers as previously described (TCRVG2PST-TCRVG12PST, and JGT_{12}-, JGT_3 and JGT_4) a discrete rearrangement has been detected in 3 of 4 normal T-cell clones.

CLAIMS:

1. A method for the detection of lymphoid leukaemia and/or lymphoma in a tissue sample, which comprises the determination of homogeneity or heterogeneity of the length of immunoglobulin and/or T-receptor gene segments in said tissue sample to indicate the presence or absence of monoclonality of the B- and/or T-lymphocyte population in the sample.

2. A method for the detection of monoclonality and presumptive malignancy in a tissue sample, and/or for the determination of the B-lymphocyte or T-lymphocyte origin of a tumour, which comprises the steps of:

(a) amplification of immunoglobulin and/or T-receptor gene segments in said tissue sample by means of the polymerase chain reaction using specific primers or mixtures of primers for said immunoglobulin and/or T-receptor gene segments; and

(b) size separation of the amplified segments to determine homogeneity or heterogeneity of the length of the amplified segments.

3. A method according to claim 2, wherein said primers are consensus primers for the immunoglobulin and/or T-receptor gene segments.

4. A method according to claim 1 or claim 2, wherein said tissue sample is selected from blood, tumour tissue in node or bone-marrow, formalin-fixed embedded histological material, aspirated cytological material or cells on slides.

5. A method according to claim 2, wherein said size separation step is selected from electrophoresis or chromatography.

6. A method according to claim 5, wherein said size separation step comprises electrophoresis on an agarose or polyacrylamide gel.

7. A method according to claim 2, wherein said size separated amplified gene segments are detected by non-isotopic means, by radioisotope incorporation, or by Southern transfer and hybridization to an internal probe.

8. A method according to claim 7, wherein said detection by non-isotopic means comprises ethidium bromide staining.

9. A method according to claim 2, wherein said primers for immunoglobulin gene segments comprise primers to the framework portions of the V regions and to parts of the D, J and/or C regions of the immunoglobulin gene.

10. A method according to claim 9, wherein said primers for immunoglobulin gene segments are selected from:

ELJ_H-5'TGAGG AGACG GTGAC CAGGG TNCCT TGGCC CCAG3'
LJ_H- 5'TGAGG AGACG GTGAC C 3'
VLJ_H-5' GTGAC CAGGG TNCCT TGGCC CCAG3'

FR1BR: 5'CT[C/G][T/A]C CTG[T/C][G/A]CAG[T/C]C TCTGG 3'
FR1BS: 5'CTCTC CTGTG CAGCC TCTGG 3'
FR2A: 5'TGG[A/G]T CCG[C/A]C AG[G/C]C[T/C]
[T/C]CNGG 3'
FR3A: 5'ACACG GC[C/T][G/C]T GTATT ACTGT 3'
FR3A-: 5'ACAGT AATAC A[G/C][A/G]GC CGTGT 3'
(where N represents any of the nucleotide bases).

11. A method according to claim 2, wherein said primers for T-receptor gene segments comprise primers to the conserved V regions and to parts of the D, J and/or C regions of the T-receptor gene.

12. A method according to claim 11 wherein said primers for T-receptor gene segments are selected from:

(a) T-receptor alpha chain primers

JAT1 5' TTC CAA AGA TCA G(T/C)T TG 3'
JAT2 5' A(A/G)C CTG GT(T/C) CCT (T/G)(T/G)T CAA A3'
3VTA 5' GA(C/T) TCA GC(T/C) GTG TAC (T/C)(T/A)(C/T) TG 3'
5VTA1 5' (A/T)AC (C/T)T(A/c) (C/T)TC TGG TA(C/T) AA(A/G) CA 3'
CTA 5' GAA TAG GCA GAC AGA CTT GT 3'
PTA 5' ACT GGA TTT AGA GTC TCT 3'

(b) T-receptor beta chain primers

JBT1 5' CG GGT (G/C)CC T(T/G)G CCC (G/A)AA 3'
JBT2 5' AG CAC GGT GAG CC(G/T) (G/T)GT (G/C)CC 3'
3VTB1 5' GAC (T/A)(C/G)A (G/A)(G/C)C GTG TAT CT(C/T) TG3'
3VTB2 5' CAG ACA TCT CTG TAC (C/T)TC TGT GCC 3'
5VTB1 5' C(A/T)(C/A)(T/C)(A/C) T (T/G)T(A/T)(T/C) TGGTA CCGAC AG 3'
5VTB2 5' TGTAC TGGTA TCGAC AAG 3'
CTB 5' TTT GGG TGT GGG AGA TCT CTG C 3'
PTB 5' CTT CTG ATG GCT CAA ACA 3'

(c) T-receptor gamma chain primers

JGT12-	5' AAG TGT TGT TCC ACT GCC AAA 3'
3VTG	5' GTC TAT TAC TGT GCC ACC TGG 3'
CTG	5' TCT GGA GCT TTG TTT CAG CAA 3'
PTG	5' GGA AGA AAA ATA GTG GGC 3'

13. A method according to claim 11, wherein said primers for T-receptor gene segments comprise a mixture of T-receptor gamma chain primers for the V- and J-regions selected from:

V-regions

Primer	Sequence
TCRVG2PST	5' CTTC CTGCAG ATG ACT CCT ACA ACT CCA AGG TTG 3'
TCRVG3PST	5' CTTC CTG CAG ATG ACG TCT CCA CCG CAA GGG ATG 3'
TCRVG4PST	5'CTTC CTG CAG ATG ACT CCT ACA CCT CCA GCG TTG 3'
TCRVG5PST	5' TTC CTG CAG ATG ACG TCT CCAA CTC AAA GGA TG 3'
TCRVG8PST	5' CTTC CTGCAG ATG ACT CCT ACA ACT CCA GGG TTC 3'
TCRVG9PST	5' GG(A/G/C/T) ACTG CAG GAA AG GAA TCTG GCATT CCG 3'
TCRVG10PST	5' CT CTG CAG AAT CCG CAG CTC GAC GCA GCA 3'
TCRVG11PST	5' CA CTG CAG GCT CAA GAT TGC TCA GGT GGG 3'
TCRVG12PST	5' ACT CTG CAG CCT CTT GGG CAC TGC TCT AAA 3'

J-regions

Primer	Sequence
JGT_{12}-	5' AAG TGT TGT TCC ACT GCC AAA 3'
JGT_3	5' AGTTA CTA TG AG C (T/C) T AGT CCC 3'
JGT_4	5' TGT AAT GAT AAG CTT TGT TCC 3'

14. A kit for performance of the method according to claim 2 which comprises a set of specific primers or mixtures of primers for immunoglobulin and/or T-receptor gene segments for amplification of immunoglobulin and/or T-receptor gene segments in a tissue sample by the polymerase chain reaction.

15. A kit according to claim 14, comprising a set of consensus primers for the immunoglobulin and/or T-receptor gene segments.

16. A kit according to claim 14, comprising primers for immunoglobulin gene segments as defined in claim 9 or claim 10.

17. A kit according to claim 14, comprising primers for T-receptor gene segments as defined in claim 11, claim 12 or claim 13.

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 6

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.[4] C12Q 1/68, C12P 19/34, C07H 21/04

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
IPC	WPI, WPIL keywords: LYMPHOCYTE; T-CELL RECEPTOR; T-RECEPTOR; T-LYMPHOCYTE RECEPTOR, IMMUNOGLOBULIN GENE AND C12Q
USPA, USPB	435/6 and LYMPHOCYTE

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 8

AU: C12Q 1/68, C12N 15/00, CHEMICAL ABSTRACTS keyword as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category*	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No 13
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P, Y	BIOTECHNIQUES, vol 7 no 5, 1989 pp 438-442, E. Levedakou et al "A strategy to study Gene Polymorphism by direct sequence analysis of cosmid clones and amplified genomic DNA"	1-17
X Y	Med Oncol & Tumor Pharmacother; vol 3 no 3/4 pp 153-157, 1986 C.I.E. Smith and L. Hammarstrom, "DNA rearrangements in human B- and T- cell malignancies"	1, 4 2, 3, 5-17

continued

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
1 February 1990 (01.02.90)	0 8 FEBRUARY 1990
International Searching Authority	Signature of Authorized Officer
Australian Patent Office	J.H. CHAN

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category*	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU 89/00455

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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END OF ANNEX